PATENT SPECIFICATION (11) 1 515 023

1 515 023

(21) Application No. 20612/75 (22) Filed 15 May 1975 (19)
(31) Convention Application No. 470 076 (32) Filed 15 May 1974 in
(33) United States of America (US)
(44) Complete Specification published 21 June 1978
(51) INT. CL.[2] G01N 21/00 B01D 15/08
(52) Index at acceptance
G2J 35
B1H 260 555

(54) PHOTOMETRIC SYSTEM

(71) We, WATERS ASSOCIATES, INCORPORATED, a corporation organised and existing under the Laws of the State of Delaware, United States of America, of Maple Street, Milford, Massachusetts, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to photometer apparatus and a process for the measurement of radiation absorptivity.

In analysis of very small quantities of liquids, it has been recognized that the physical conditioning of the fluid must be done very carefully. Thus, for example, in the field of liquid chromatography wherein very small, continuously-flowing streams of liquid are measured, care is taken to minimize mechanical and thermal disurbance of the liquid stream between the chromatographic column and analytical apparatus in which the liquid stream from the column is to be continuously analyzed. The primary objective is to present, to a transparent sample cell, the precise sequence of changing liquid composition that leaves the chromatography column.

The rationale and particulars of such apparatus are described in the art. For example, see U.S. Patent 3,674,373 to Waters, Hutchins and Abrahams which involves a refractometer particularly well adapted to receive such a liquid stream. In general, the approach is to minimize the conduit path through which the liquid to be analyzed must travel and to provide a maximum thermal-conditioning of the liquid within such a minimized path. This generally illustrates the art-recognized importance of careful handling of sample liquid between its point of origin and the sample cell in which it is to be subjected to analysis, usually analysis which measures an effect of the sample liquid stream on some radiation directed into a flow-cell through which the stream passes.

Investigators have also realized that some attention must be given to the physical condition of the fluid even *after* it enters the flow-cell. Consequently, flow-cells have been made ever smaller to avoid mixing and peak-spreading effects and, in some cases, a positive thermal equilibration of the cell with the liquid has been sought in order to avoid light-shimmering effects along the cell walls. Moreover, the cells are usually positioned with outlets so placed that any entrained gas bubbles tend to be carried upwardly out of the cell. It is noted that U.S. Patent 3,666,941 to Watson describes a conical bifurcated cell wherein the larger end of the cell faces the light source, thereby forming means to gather a maximum amount of fluorescence-exciting radiation. Applicant's discovery, to be detailed below, is based upon a major improvement in flow-cell construction which solves a problem quite different than that described by Watson but which, like Watson's apparatus, is particularly useful in combination with liquid chromotography applications.

A recent patent, U.S. Patent 3,792,929, to Alpert, it has been noted, seems to disclose a conical sample-holding cell. The patent relates to static-sample devices and in no way involves fluid lenses of any type; although the patent came to the attention of the instant inventor after an error resulted in the word "field" appearing as "fluid" in the title of the Alpert patent. Moreover, the apparent and relative dimensions of the Alpert cell would not allow its effective use in most continuous-flow monitoring systems such as are encountered in liquid chromatographic work and the like.

According to one aspect of the invention there is provided a photometer employing a radiation source and including a sample cell having a radiation inlet port and a radiation outlet port, the cell being so designed that a continuously-flowing liquid to be analysed can be passed from a region adjacent the inlet port through a flow path to a region adjacent the outlet port whereby radiation from the source can enter the cell via the inlet port, and means for measuring the absorption of radiation from the source in the sample cell, the inlet port aperture being smaller than the outlet port aperture

and the cross-section of the cell becoming progressively larger from the inlet port to the outlet port, whereby the lens effect of the liquid is minimised and loss of radiation due to refraction on to the walls of the cell is substantially reduced.

According to another aspect of the invention there is provided a process for measuring the radiation absorptivity of a flowing liquid sample which comprises a plurality of sequential liquid compositions in a laminar flow mode, the improvement comprising minimising the interference of a dynamic liquid lens with said measuring by

a. feeding said liquid into a sample cell near to one end, the cell having a radiation inlet aperture at the one end which is smaller than a radiation outlet aperture at the other end and a cross-section which becomes progressively larger from the inlet aperture to the outlet aperture,

b. removing said liquid from said sample cell near to the other end thereof,

c. and measuring the radiation absorptivity of said liquid passing through said cell, said measurement being carried out by detecting substantially all of the non-absorbed radiation which passes from a source into the cell via the smaller aperture and leaves the cell via the larger aperture.

According to yet a further aspect of the invention there is provided a liquid chromatographic analytical apparatus of the type having a liquid chromatographic column adapted to emit a liquid stream comprising a series of sequentially-arranged liquid compositions, and means for conducting said stream to a photometer of the type comprising a sample flow-cell forming a conduit for said liquid stream, means to provide a source of radiation, and a radiation detector arranged with respect to said conduit to form a radiation path therethrough, wherein said radiation detector includes means to receive substantially all non-absorbed light transmitted through said sample cell, said apparatus further including means for minimising distortion of said radiation by dynamic liquid lens effects wherein the flow-cell has a radiation inlet aperture at one end which is smaller than a radiation outlet aperture at the other end and a cross-section which becomes progressively larger from the one end to the other end, the radiation being arranged to pass from the source into the cell via the aperture at the one end and out of the cell at the other end to the detector means, such that any loss of refracted radiation on walls of said flow cell is minimised.

The above invention is based on the discovery that substantial spurious radiation signals are generated by a lens-type effect caused by laminar-flow patterns at the interface of compositions differing in refractive index; the effect is particularly troublesome in small cylindrical photometer sample-cells. These laminar flow patterns will sometimes be called "dynamic liquid lenses" in this description. In general the worst problems have been encountered in flow-cells in the microliter range, say flow-cells having a diameter of less than about 2 millimeters. In the usual situation the flow path of an ultra violet absorptometer cell is selected to be one centimeter in length, and a flow cell of 2 millimeters maximum diameter will have a volume of less than about 32 microliters. As the diameter increases the lens effect caused by a given rate of laminar-flow tends to decrease; but a mere increase in diameter of a cylindrical flow path to avoid the lens effect is not practical because the increased diameter would result in either (1) a large increase in the volume of the tube or (2) a substantial decrease in length of the tube. A large increase in volume is untenable because the ability of the apparatus to detect very small samples would be substantially limited by dilution factors. The length of the cell cannot be markedly reduced without proportionately decreasing the magnitude of light absorbed by a given solution flowing through a cell. Still other conceivable tube configurations would give disadvantageous liquid flow patterns.

Because the problem of these dynamic fluid lenses is primarily encountered at the point of changing compositions, its solution has been found to enhance both the quantitative and qualitative analytical capabilities of liquid chromatographic systems and like analytical systems where constantly changing compositions are inherent in the method. However, the apparatus is useful in other lens-inducing situations encountered in the process industry; e.g., where the dynamic fluid lens may be induced by temperature change or other phenomena that result in formation of a refractive index gradient within the flow-cell.

The present invention provides a simple constructional solution as a result of which the lens effect in a flow cell is rapidly dissipated by a progressive increase in the cross-sectional area of the flow-cell along the flow path. Thus, the wall of the flow-cell advantageously forms a diverging surface of rotation whereby the wall form an angle of divergence of at least about one angular degree with the axis of the cell. An angle of about 1.5 or slightly greater provides sufficient widening to substantially dissipate the undesirable effect of the dynamic liquid lens formed at the interface of water and most organic solvents. The improvement is largely achieved by collecting refracted light, which would have otherwise been absorbed on the wall of the cell, but it is also believed the

reducion in velocity of the stream during its transit through the cell—usually a reduction of over 50%—causes a dissipation of the lens effect itself which reduces the amount of refracted light directed against the walls of the cell. Angles of divergence between the axis of the flowpath and the wall of the cell of 1° to 3° are most advantageous; larger angles only become problems because they usually dictate a larger cell size.

In liquid chromatographic applications, best results will be achieved if the apparatus to be used with the flow-cell is selected to achieve the most ideal flow pattern possible. This is true of all flow in a liquid chromatographic system: flow from sample injection to a column and flow between the column and the analytical component of the system. Such apparatus is available: an injector advantageously used is that available under the trade description Model U6K Injector by Waters Associates, Inc. A pumping system, advantageously used to feed liquid into a high pressure column, is that available from the same source under the trade designation Model 6000 Solvent Delivery System. However, as will be obvious to those skilled in the art, other such apparatus will be generally useful in many applications in which the present invention is advantageously used.

It will also be obvious to those skilled in the art that a number of modifications can be made in the shape of the wall structure of the flow-cell. For example, further enlargement of the cell conduit over that defined minimal conical shape will yield an operable cell that will avoid the effect of the dynamic liquid lens but will also be larger in size and therefore less favorable for many applications. Such enlargement is nonfunctional with respect to the present invention. However other such shapes, for example catenoidal horn, hyperbolic horn and parabolic surfaces as well as similar surfaces of revolution can be used in the performance of the invention and are all intended to be covered by the term "generally truncated cone" as used in this application. Such shapes may on some occasions be favorable in view of effects caused by special flow properties of the fluid components which form the dynamic lens, temperature profiles across the cell, friction effects along the surface of the wall or the like. "Generally conical", therefore, is meant to include any flow-cell wherein the aperture of the inlet port for the radiation is smaller than the aperture of the outlet port for the radiation and the cross section of the cell is progressively larger as measured closer to the outlet port.

It is to be realized that the most important structural aspect of the invention relates to the relationship of the conical cell to the direction of the lightpath: the larger end of the cone must be toward the detector. It is possible, however, to reverse the direction of flow of the liquid to be analyzed through the cell. Best practice is to avoid this situation or, if for some reason it is desirable, to arrange the attitude of the cell so that any minute gas bubbles canbe displaced upwardly toward the outlet of the cell.

In chromatographic related analytical operations and other such operations which monitor microliter quantities of a flowing sample, the length-to-average diameter ratio of the flow cell is advantageously at least 5 to 1. It is primarily the monitoring of such small samples, rather than inherent optical considerations, which make angles of divergence greater than 3° undesirable for many applications.

One additional advantage of the apparatus disclosed herein is the fact that, for some applications, it allows the light source to be brought (physically, or by optical means) closer to the sample cell without undue losses of light by refraction and light scattering occurring primarily at the interfaces of gas-lens and liquid-lens interfaces.

In this application and accompanying drawings there is shown and described a preferred embodiment of the invention and suggested various alternatives and modifications thereof, but it is to be understood that these are not intended to be exhaustive and that other changes and modifications can be made within the scope of the invention. These suggestions are selected and included for purposes of illustration in order that others skilled in the art will more fully understand the invention and the principles thereof and will be able to modify it in a variety of forms, each as may be best suited in the condition of a particular case.

In the drawings:

Figure 1 is a schematic diagram of an analytical apparatus constructed according to the invention.

Figure 2 is a section of a flow-cell constructed according to the invention.

Figure 3 is a graph illustrating the output signal of an ultra-violet absorption-measuring apparatus using a conventional cylindrical flow-cell.

Figure 4 is a graph illustrating a chart similar to that shown in Figure 3 but obtained utilizing a flow-cell constructed according to the invention.

Figure 1 illustrates an analytical system 10 comprising a source 12 of a liquid to be analyzed, a liquid chromatography column 14, and an ultra-violet absorptometer 16 comprising a light source 18, an interference filter 20, a lens system 22 providing a parallel light beam, front windows 23, main housing wall of a sample cell 24, a rear window 26 and photoelectric detector 28. Signals

from photo detector 28 and a reference detector 28a are processed according to known techniques to provide a suitable electronic signal which may be used as a control means or as is more frequent, to provide a visible recording on a recorder means 30.

The single novel feature in Figure 1 is the sample cell 24 which incorporates the conical flowpath 32. However, this innovation directly enhances the performance of the entire system by providing means to take the liquid output from chromatographic column 14 and process it in the ultra-violet absorption apparatus so that the resulting light reaching detector 28 is substantially free of detrimental loss of light due to the influence of dynamic liquid lenses.

In the apparatus of Figure 1, the light source is rated at 2.4 watts and has principal wave length of 253.7 nanometers. The volume of the sample cell, best seen in Figure 2, is about 12.5 microliters: it is about 0.04 inches in diameter at the inlet end, about 0.06 inches in diameter at the outlet end and about 0.394 inches in length. A reference flow-cell 34 is positioned within cell assembly 36, as is common in the photometric analysis of liquids. This cell may be empty, full of a stagnant liquid or have a flowing reference fluid therein.

Figure 3 illustrates graphically the type of detection problem which can be encountered in radiation-absorption analysis because of interference in ultra-violet transmittance by dynamic liquid lenses as they move through a thin cylindrical sample cell.

In each of Figures 3 and 4, there is an initial peak 60 caused by a calibration fluid —a standard dichromate solution flowing through the cells at a rate of one milliliter per minute. The next rise 61 in each curve, is merely an adjustment of the zero level of the recorder. At this point, each curve has a relatively flat reference level indicative of the low ultra-violet absorption of water.

This reference level is flat for the continuous feed in Figure 3 but interrupted by abrupt drops in light transmission when injections of aqueous methanol solution are introduced into the column. These apparent increases in absorption are caused by the refraction from dynamic fluid lens formed by the methanol-water interface and the interfaces of various mixtures thereof. Once refracted, a substantial portion of light is absorbed on the parallel walls of the conventional flow-cell.

The valleys 64 of Figure 3 illustrate the effect caused by a transition from water flow of .3 ml/minute to a flow of 0.3 ml per minute of a 10% aqueous solution of methanol. This solution is added through a sample loop over a period of about 3.3 minutes. Then, as water returns flushing the loop, there is an upward displacement 65 of the curve caused by the dynamic liquid lens now being formed by the water flush flowing behind the methanol solution. After the flushing with water is completed, fluid-lens induced displacement subsides until another injection of water-methanol solution is started.

Equivalent injections made in the same system, except for the use of a flow-cell as shown in Figure 2 result in no reduction in transmission, when methanol is added. Nor is there any substantial increase in transmission when the water flush occurs. Such points are identified as 64a and 65a in Figure 4.

It is stressed that it is intended to cover the apparatus of the invention as claimed, whether it exists as assembled or non-assembled parts.

WHAT WE CLAIM IS:—

1. A photometer employing a radiation source and including a sample cell having a radiation inlet port and a radiation outlet port, the cell being so designed that a continuously-flowing liquid to be analysed can be passed from a region adjacent the inlet port through a flow path to a region adjacent the outlet port whereby radiation from the source can enter the cell via the inlet port, and means for measuring the absorption of radiation from the source in the sample cell, the inlet port aperture being smaller than the outlet port aperture and the cross-section of the cell becoming progressively larger from the inlet port to the outlet port, whereby the lens effect of the liquid is minimised and loss of radiation due to refraction onto the wall of the cell is substantially reduced.

2. A photometer as claimed in claim 1 wherein said light source and said measuring means are so selected that said photometer is an ultra-violet absorbance detector.

3. A photomether as claimed in either claim 1 or claim 2 wherein an angle of divergence between an axis of said flowpath and the wall of said flow-cell is from 1° to 3°.

4. A photometer as claimed in any one of the preceding claims wherein said sample cell has a volume of less than 32 microliters and a maximum diameter of less than 2 millimeters.

5. A photometer as claimed in any one of the preceding claims wherein the length to average diameter ratio of the flowpath is at least 5:1.

6. A process for measuring the radiation absorptivity of a flowing liquid sample which comprises a plurality of sequential liquid compositions in a laminar flow mode, the improvement comprising minimising the interference of a dynamic liquid lens with said measuring by

a. feeding said liquid into a sample cell near to one end, the cell having a radiation inlet aperture at the one end which is smaller than a radiation outlet aperture at the other end and a cross-section which becomes progressively larger from the inlet aperture to the outlet aperture,

b. removing said liquid from said sample cell near to the other end thereof,

c. and measuring the radiation absorptivity of said liquid passing through said cell, said measurement being carried out by detecting substantially all of the non-absorbed radiation which passes from a source into the cell via the smaller aperture and leaves the cell via the larger aperture.

7. A process as claimed in claim 6, wherein the radiation being measured is ultra-violet light.

8. A process as claimed in either claim 6 or claim 7 wherein the velocity of the sample liquid is decreased by at least 50 percent during is movement from the one end of said sample cell to the other end of said cell.

9. A process as claimed in any one of claims 6 to 8 wherein the volume of liquid sample in said flow-cell is maintained at less than about 32 microliters and wherein said maximum diameter of said cell is 2 millimeters.

10. A liquid chromatographic analytical apparatus of the type having a liquid chromatographic column adapted to emit a liquid stream comprising a series of sequentially-arranged liquid compositions, and means for conducting said stream to a photometer of the type comprising a sample flow-cell forming a conduit for said liquid stream, means to provide a source of radiation, and a radiation detector arranged with respect to said conduit to form a radiation path therethrough, wherein said radiation detector includes means to receive substantially all non-absorbed light transmitted through said sample cell, said apparatus further including means for minimising distortion of said radiation by dynamic liquid lens effects wherein the flow-cell has a radiation inlet aperture at one end which is smaller than a radiation outlet aperture at the other end and a cross-section which becomes progressively larger from the one end to the other end, the radiation being arranged to pass from the source into the cell via the aperture at the one end and out of the cell at the other end to the detector means, such that any loss of refracted radiation on walls of said flow cell is minimised.

11. A chromatographic apparatus as claimed in claim 10 wherein said source and said detecting means are so selected that said radiation detector is an ultraviolet radition detector.

12. A chromatographic apparatus as claimed in either claim 10 or 11 wherein said flow-cell has a maximum diameter of 2 millimeters.

13. A chromatographic apparatus as claimed in any one of claims 10—12 wherein the angle of divergence between the conical wall and axis of said flowpath is from 1° to 3°.

14. Apparatus as claimed in any one of claims 10—13 wherein said flowpath has a length-to-average diameter ratio of at least 5:1.

15. A photometer as claimed in claim 1 substantially as described herein with reference to Figures 1 and 2 of the accompanying drawings.

16. A method of operating a photometer as claimed in claim 15 substantially as described herein with reference to Figure 1 of the accompanying drawings.

JOHN ORCHARD & CO.,
Chartered Patent Agents,
Staple Inn Buildings North,
High Holborn.
London, WC1V 7PZ.
Agents for the Applicants.

Printed for Her Majesty's Stationery Office by Burgess & Son (Abingdon), Ltd.—1978.
Published at The Patent Office, 25 Southampton Buildings, London, WC2A 1AY,
from which copies may be obtained.

1515023 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 1

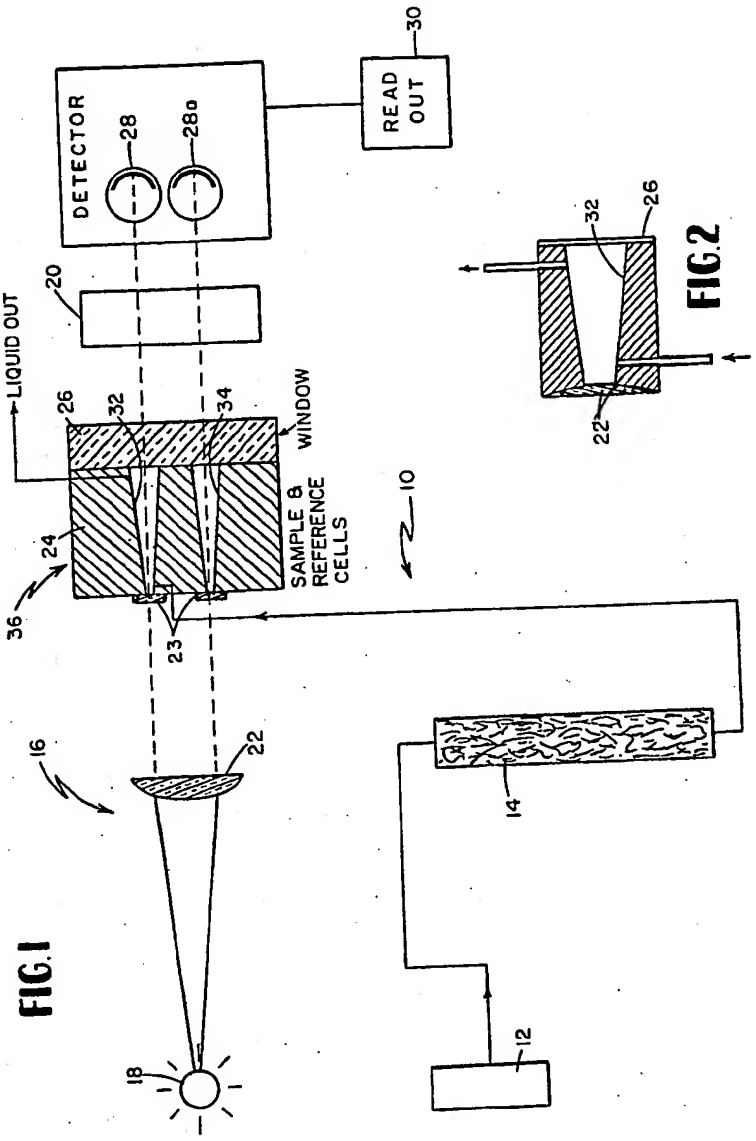

FIG.1

FIG.2

1515023 COMPLETE SPECIFICATION
2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*
Sheet 2

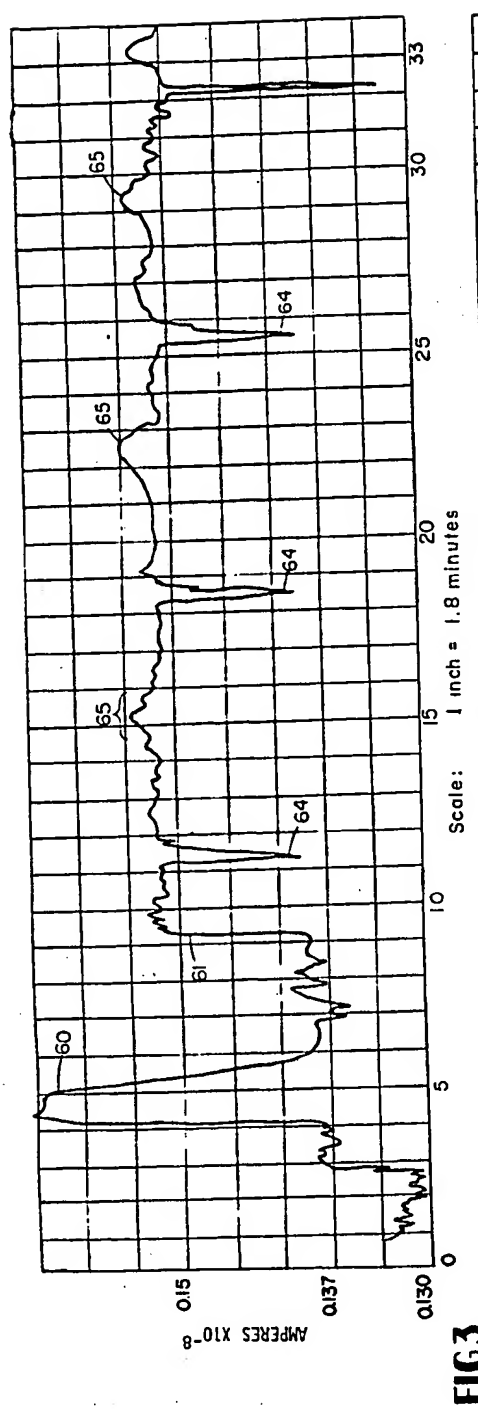

FIG.3

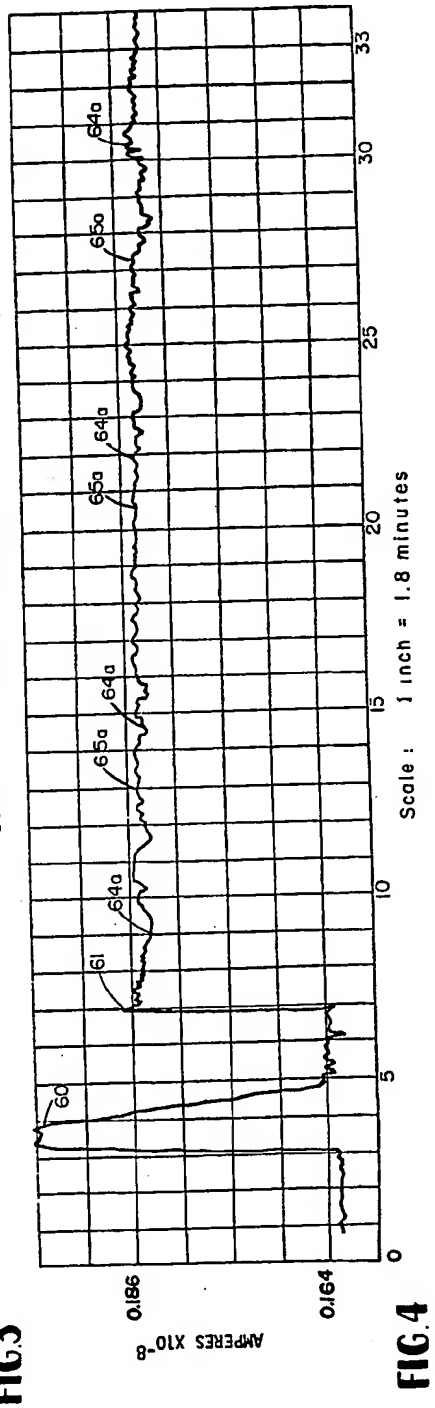

FIG.4